AD_____

Award Number: DAMD17-03-1-0473

TITLE: Stromal gene expression and function in primary breast tumors that metastasize to bone cancer

PRINCIPAL INVESTIGATOR: Belinda Parker
Robin L. Anderson

CONTRACTING ORGANIZATION: University of Melbourne
Victoria 3010 Australia

REPORT DATE: July 2004

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

# REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188



| 1. AGENCY USE ONLY (Leave blank) | 2. REPORT DATE | 3. REPORT TYPE AND DATES COVERED |
|---|---|---|
| | July 2004 | Annual Summary (1 Jul 03-30 Jun 04) |

**4. TITLE AND SUBTITLE**
Stromal gene expression and function in primary breast tumors that metastasize to bone cancer

**5. FUNDING NUMBERS**
DAMD17-03-1-0473

**6. AUTHOR(S)**
Belinda Parker
Robin L. Anderson

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**
University of Melbourne
Victoria 3010 Australia

E-Mail: Belinda.parker@petermac.org

**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

**10. SPONSORING / MONITORING AGENCY REPORT NUMBER**

**11. SUPPLEMENTARY NOTES**

**12a. DISTRIBUTION / AVAILABILITY STATEMENT**
Approved for Public Release; Distribution Unlimited

**12b. DISTRIBUTION CODE**

**13. Abstract** ***(Maximum 200 Words):*** A clinically relevant syngeneic model of breast cancer metastasis has been used to determine gene expression alterations that occur both between primary breast cancers with varying metastatic potential and between matched primary and bone metastases. We have immunopurified epithelial and endothelial cell populations and profiled them separately to identify differentially expressed genes, some of which have not previously been associated with breast cancer metastasis. Expression profiles of vascular endothelium derived from primary tumors of varying metastatic potential identified aberrant expression of genes involved in angiogenesis, cell cycle progression, cytoskeletal structure and tumor suppression. Those altered in the primary tumor epithelium included developmental genes, metastasis suppressors and genes involved in cytoskeletal organization, cell cycle progression, apoptosis and transformation. The microarray data was confirmed by quantitative RT-QPCR.

Further analysis of epithelium from matched spine metastases revealed some genes that were up-regulated further at the metastatic site. These included stefin A1 (inhibitor of cathepsin S) that was up-regulated in highly metastatic primary epithelium and increased a further 9-fold in matched bone metastases. The expression in spine metastases was verified by *in situ* hybridisation whilst the expression of stefin A1 in subsets of tumor cells in invasive human breast cancer was confirmed by immunohistochemistry.

**14. SUBJECT TERMS**
No subject terms provided.

**15. NUMBER OF PAGES**
26

**16. PRICE CODE**

| 17. SECURITY CLASSIFICATION OF REPORT | 18. SECURITY CLASSIFICATION OF THIS PAGE | 19. SECURITY CLASSIFICATION OF ABSTRACT | 20. LIMITATION OF ABSTRACT |
|---|---|---|---|
| Unclassified | Unclassified | Unclassified | Unlimited |

## Table of Contents

## INTRODUCTION

Breast cancer is a high frequency disease that can often be treated successfully if detected early, yet once metastasis occurs there is a reduced chance of patient survival. A large proportion (~70%) of patients who die of their disease have skeletal involvement (1), indicating that bone metastasis is a major cause of morbidity in patients with breast cancer. Due to the high frequency and severity of breast cancer metastasis to bone, and the lack of markers that allow for early detection and therapy, it is necessary to determine the cellular and molecular mechanisms by which the specific spread of breast cancer cells to the bone occurs. There are a number of steps in the metastatic process, including local invasion of breast cancer cells out of the stroma and the ability to overcome cell-cell interactions to enter the circulation and the subsequent interaction between the cancer cell and the secondary host environment to stimulate metastatic tumor growth. The steps involved in tumor progression is influenced by both tumor and stromal genes, with recent evidence revealing that stimulation of host stromal genes is important in breast cancer progression (2-4). Identification of the genes critical to allowing specific growth in bone and other metastatic sites is important for the development of therapeutic strategies.

Although many genes have been found to be associated with breast cancer metastasis, a single accurate predictor to identify patients who will develop bone disease is still lacking. This is largely due to the lack of clinically relevant models of breast carcinoma metastasis to bone to evaluate the function of genes already implicated in the metastatic process and to find new gene candidates. Models used to find these genes have usually relied on cell culture or xenograft models of bone metastases in the absence of primary breast tumor formation (eg. MDA-MB-231 injected into left ventricle of nude mice). This makes it difficult to study the steps of metastatic progression from the initial invasion of cells into the circulation to the growth in the bone microenvironment. Also, whole tumor gene expression analysis ignores the contribution of tumor-associated stromal cells to growth and invasion of tumor cells. The use of cell specific profiling may therefore identify gene candidates in stromal cells that could have been masked using whole tumor analysis. A recent study reported major alterations in gene expression of breast cancer associated myoepithelium (5), changes that have not been identified in past studies.

A recent spontaneous metastasis model has been developed in our Laboratory. This model mimics the clinical disease in that a primary breast tumor develops, the cells invade through the stroma into the circulation and colonize at a distant organs (Figure 1) (6). This is the first reported model of spontaneous metastasis of breast tumor cells from the primary site in the mammary gland to different organs, including bone. The model is of enormous importance for a comparison of the expression profiles of primary tumors with different metastatic capacities. This proposal aims to determine cell-specific expression changes during breast cancer metastatic progression. The model can then be used for functional analysis of genes identified to determine whether they are in fact involved in the metastatic process and whether they have therapeutic potential.

The main objectives are to study the molecular events involved in metastasis, specifically we aim to 1) identify genes expressed in tumor-derived endothelial cells that may be associated with metastasis; 2) determine whether these genes are also expressed in human breast cancer, 3) study the function of these genes using *in vitro* metastasis and angiogenesis assays; 4) explore the role of these genes in metastasis to bone *in vivo.*

## BODY

*TASK 1: Isolate tumor and stromal cells from primary tumors of differing metastatic capacity* and subject the RNA isolated from these cells to microarray analysis *(months 1-12)*

a. Purify specific cell populations (epithelial, endothelial and fibroblast cells) using immunopurification from fresh tumors and by laser capture microdissection (LCM) from frozen tumors (months 1-9).
b. Perform microarray analysis of specific cell types, comparing RNA from cells derived from a non-metastatic tumor to that from a metastatic tumor (months 6-12).

Task 1 has now been completed. We have separated epithelial cells, endothelial cells and remaining stromal cells from the primary tumors of the tumor lines shown in Figure 1. The separation was done individually for six tumors of each line following 28 days growth in the mammary gland of Balb/c mice. Fresh resected tissue (normal fat pad, primary tumor tissue or the metastatic sites spine, femur and lung) was obtained and cell separation completed using an immunobead protocol adapted from Vogelstein and colleagues (7). Purity of the epithelial and endothelial cell populations was confirmed by immunostaining for carcinoembryonic antigen (CEA) and von Willibrand factor (vWF), respectively and also by RT-PCR of epithelial (CK18) and endothelial (vWF, P1H12) associated genes (Figure 2). During the cell isolation procedure, epithelial cells, endothelial cells, B and T cells and red blood cells are removed. The composition of the "remaining stromal cells" is likely to be primarily fibroblasts, with a small number of other cells including macrophages and adipocytes likely to be present.

Amplified RNA isolated from epithelial and endothelial cells was used for cDNA microarray analysis using the NIA mouse 15K custom array (Microarray Facility, Peter MacCallum Cancer Centre) with newborn mouse RNA as a reference. Microarray results were analysed using Genepix and Genespring v.6 software and gene expression profiles of epithelial and endothelial samples were compared to determine those that were significantly different ($p<0.05$) in high metastatic samples compared to low metastatic and normal samples, by at least 1.8 fold. It should be noted that amplification of RNA did not produce any bias as confirmed by the lack of significant difference between expression profiles of the same sample that was or was not amplified (data not shown).

Gene expression profiles have been obtained from the tumor epithelial and endothelial cells within the primary tumors. For each cell type, a set of genes have been identified whose expression is different between the highly aggressive bone metastasizing lines (4T1.2 and 4T1.13) and the poorly metastatic lines (66cl4 and 67NR); and in endothelial comparisons also different from that in the normal mammary gland. We have also isolated RNA from the remaining stromal cell component of each of the six primary tumors of each tumor line in the metastasis model in preparation for gene expression profiling of this cell population. Further, we have isolated RNA from the three different cell populations within metastases, where they occur, in preparation for cDNA microarray anaysis of lung and bone metastases.

The genes identified aberrantly expressed in highly metastatic tumor-associated endothelium are listed in Table 1 and include, not surprisingly, genes involved in angiogenesis, proliferation, adhesion and motility. Also of interest are some genes whose expression is suppressed in the

endothelial cells of the highly metastatic tumors, including two well known tumor suppressor genes, PTEN and LKB1. There are also genes involved in, or regulated by the Hedgehog signalling pathway: Lasp1, CREBBP/EP300 inhibitory protein 1 and FoxP1. Of interest as well are a number of differentially regulated ESTs, shown in Table 2. Interestingly, one EST down-regulated in highly metastatic tumor endothelium has recently been identified as a homolog of large tumor suppressor 2 (LATS2), again revealing endothelial-cell specific decrease in expression of tumor suppressor genes.
Some of the genes in Table 1 have been linked previously to cancer, but many have not yet either been associated with cancer or even reported before in endothelial cells. It is intended that the function of some of these genes will be examined in tumor associated endothelial cells using our metastasis model. Those of specific interest include serine/threonine kinase 11 (Stk11 or LKB1), Forkhead box P1 (FoxP1) and macrophage migration inhibitory factor (MIF).

Genes found altered in highly metastatic primary tumor epithelium are summarised in Table 3. Upregulated genes include those involved in cellular proliferation and apoptotic inhibition (DACH1), cytoskeletal organization and development (Dppa5, Mater) whereas those found to be decreased in expression in highly metastatic tumor cells include differentiation and apoptosis genes (BMP4) and metastasis suppressor genes (BRMS1). A number of ESTs were also identified as aberrantly expressed and a selection validated in Task 2.
The genes of interest for further study are listed in Table 4, along with their chromosomal localization, previous loss of heterozygosity (LOH) or gene amplification documented for that region and associated functions that may indicate a role in metastasis. It should be noted that these genes are yet to be studied for chromosomal and epigenetic aberrations and it will be of future interest to do so in our future studies (ie DNA amplification, promoter methylation for up-regulated and down-regulated genes respectively).

*TASK 2 Verify expression of differentially expressed genes found in the mouse model in the relevant cells of human breast tumors, using immunohistochemistry or in situ hybridization (months 9-18).*

a. Use realtime RT-PCR and immunohistochemistry in cell culture and in tissue sections of the mouse model to confirm the microarray data (months 9-15).
b. Confirm that these genes are also relevant to breast cancer metastasis to bone in humans by using human tissue arrays to measure expression of the identified genes in the relevant human cell type (endothelial, fibroblast or epithelial) (months 12-18).

As reported under Task 1, we have generated gene expression profiles of isolated epithelial cells from tumors that are highly metastatic compared to those that are weakly metastatic or non-metastatic. We have used our metastasis model in which spontaneous metastasis to various sites, including bone, occurs following growth of a tumor in the mammary gland. In Table 1, 2 and 3 we reported some of the genes we found to be aberrantly expressed in tumor epithelial cells and in associated host endothelium. The differences in expression for several of these genes have now be confirmed by real time quantitative RT-PCR. From the lists of epithelial genes altered in highly metastatic primary breast cancer (table 3), we have compared the expression of BMP4, Dach1 and two ESTs – NM028729 and BC042445 as shown in Figure 3. Of interest is the decreasing expression of BMP4 with increasing metastatic capacity and the reverse response for Dach1. BMP4 is a member of the TGFβ family, has a role in development, induces senescence

and is a negative regulator of Dach1, which stimulates proliferation and inhibits TGFβ induced apoptosis. Functional analysis of this interaction will be further studied *in vitro*.

Stefin A1 was also found to be expressed at much higher levels in the highly metastatic 4T1.2 and 4T1.13 primary tumor epithelium (Table 3). Stefin A1 has been reported to be an inhibitor of cathepsin S and a marker of malignancy in some tumor systems. When comparing expression profiles of epithelium isolated from primary tumors and from matched spine metastases (from 4T1.2 and 4T1.13 sublines), stefin A1 had even higher levels of expression in the bone metastases compared to the primary tumor, suggesting an important role in metastasis to bone and the possibility that only a subset of cells in the primary tumor express the gene and these cells are selected for in the bone metastases. The expression patterns were validated by RT-PCR (Figure 4) of cDNA samples from immunopurified epithelial populations derived from both the primary tumor and matched spine metastases. These results suggest that Stefin A1 has potential not only as a prognostic marker at the primary site, but also as a target for treatment of metastatic cancer since its expression is maintained (and enhanced) in bone metastases.

To determine whether Stefin A1 expression was relevant in human cancer (and hence that these studies are clinically relevant) immunohistochemistry was carried out on human primary tumors using an anti-human stefin A antibody. Interestingly, the hypothesis that only a subset of cells expressed Stefin A (and hence the relatively low expression in primary tumors compared to spine and femur metastases) held true, with only 1-2 of 6 primary samples analyzed being positive for stefin A and of those tumors only a subset of tumor cells expressed stefin A at the protein level (Figure 5). It should be noted that this antibody was not reactive to murine stefin A and therefore could not be used for verification in the mouse tumor tissues. *In situ* hybridization will be used to detect the expression of stefin A1 in primary and metastatic tumors of the mouse model.

We have also verified the microarray data derived from primary tumor vascular endothelium, before proceeding to a study of the expression of the genes *in situ* in tumors. Expression of FoxP1, LKB1, MIF, LATS2 and Snail by real time quantitative RT-PCR is shown in Figure 6. With increasing metastatic capacity, endothelial expression of FoxP1, LKB-1 and LATS2 decreased. On the other hand, there was a trend toward increased expression of MIF and SNAIL in the endothelium of highly metastatic 4T1.2 tumors. SNAIL has been shown in a previous study to be up-regulated in human breast tumor endothelium compared to normal endothelium (8).

Since a group of genes have now been verified from the microarray studies by RT-PCR on immunopurified samples, *in situ* hybridisation is now being optimised to verify histologically the cell specific expression of these genes of interest and their expression both in the primary tumors and also in metastatic lesions. Due to the lack of antibodies available, riboprobes are being made by cloning 2-3 ~400 bp cDNA fragments of each gene into pGEM Teasy and utilizing the T7 and Sp6 bidirectional promoters for generating anti-sense and sense RNA probes. The method is being optimised using the vascular endothelial specific gene KDR (VEGFR2). Constructs have already been made for KDR, FoxP1, SNAIL and LATS2. Therefore studies in the near future will be concentrating on the endothelial genes of interest, in order to determine the best candidates to take on to functional studies.

*TASK 3 In vitro* functional analysis of the selected candidates (months 18-36).

a. Perform *in vitro* invasion and migration assays using tumor cells co-cultured with endothelial cells or fibroblasts isolated from primary tumors with known metastatic potential (months 18-24).
b. Generate endothelial cells or fibroblasts transiently infected with a retrovirus expressing a cDNA construct for one of the genes of interest. Use these cells in the invasion and migration assays described above with tumor cells of varying invasive potential (months 18-36).

*TASK 4: Explore the function of the selected genes in metastasis in vivo (months 18-36).*

a. If available, obtain mice null for the stromal gene of interest. Backcross onto a Balb/c background (months 18-30).
b. Measure the metastatic capacity of the bone metastasizing clone in mice lacking the relevant stromal gene (months 30-36).
c. In normal Balb/c mice, use neutralizing antibodies, an antagonist or a small molecule inhibitor of the gene if interest to measure the effect on bone metastasis (months 18-36).

As expected by the timeline, functional studies (Task 3 and 4) have not yet been carried out for the genes of interest in Task 2. Stefin A1 is currently being cloned in preparation for retroviral infection into 67NR, 66cl4 and 4T1.2 lines. This gene will the be studied for a role in metastasis *in vitro* and in the metastasis model *in vivo*. Upon verification by *in situ* hybridisation, a number of genes derived from vascular endothelial studies will be cloned and studied functionally as per Task 3 and of the proposal.

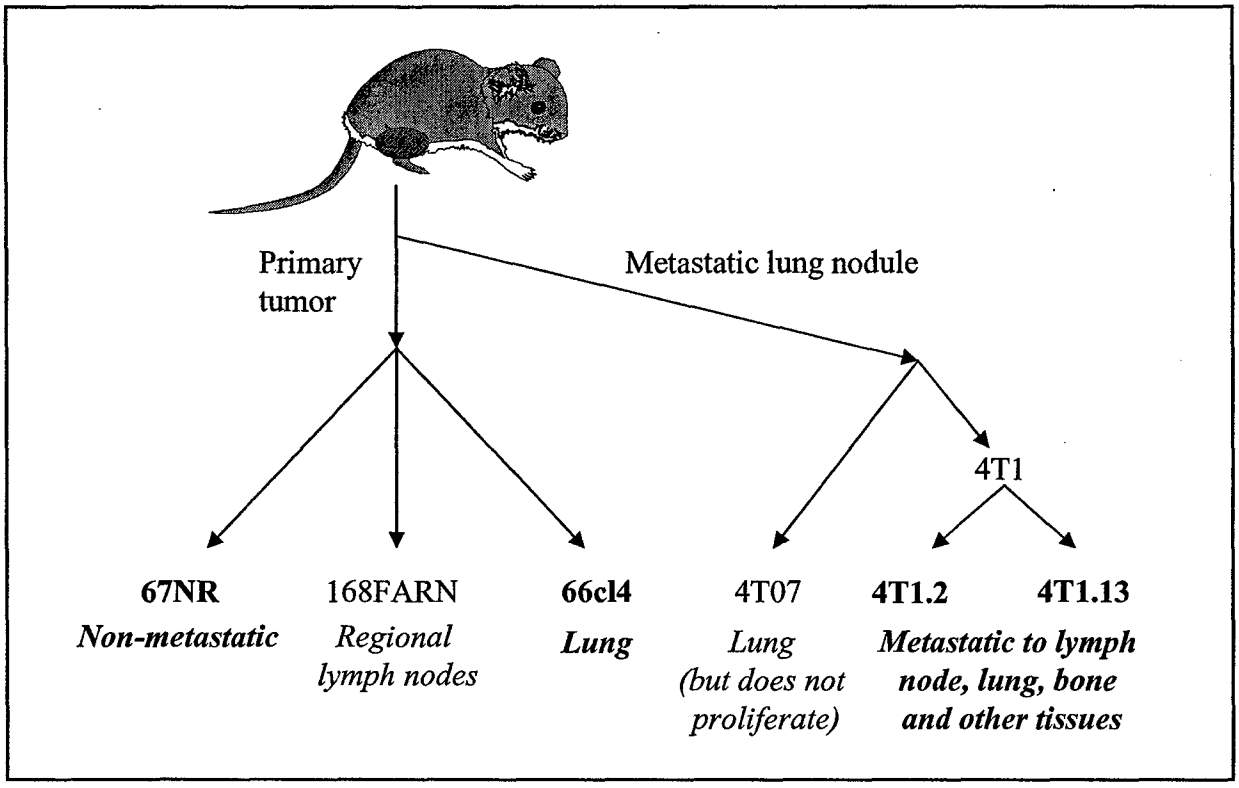
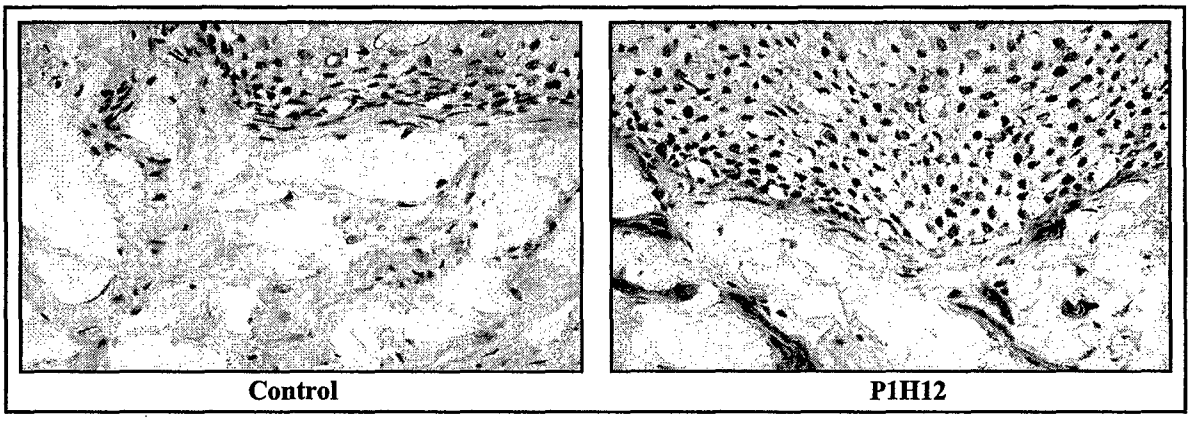
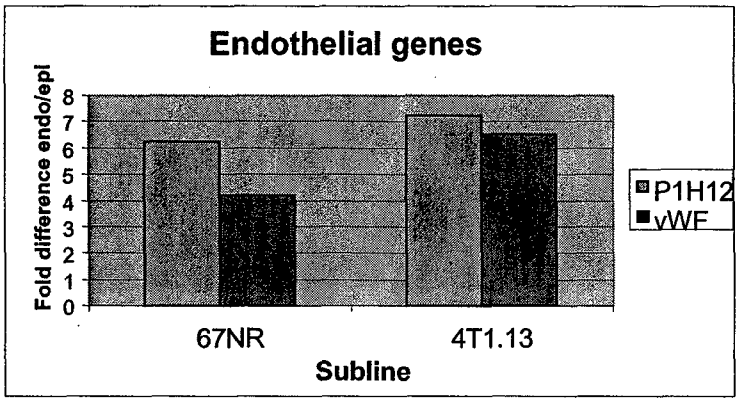



***Figure 1. Orthotopic model of breast cancer metastasis to bone.*** Several tumor sublines have been isolated from a spontaneously arising mammary gland carcinoma. Each subline has a distinct metastatic phenotype. 67NR is non-metastatic, while 168FARN, 66cl4 and 4T07 are weakly metastatic and have a tissue restricted metastatic distribution. 4T1.2 and 4T1.13 are two bone metastasizing tumor clones derived from the lung metastasizing 4T1 subline. Sublines in **bold** are those that were included in the analysis

A




B




C

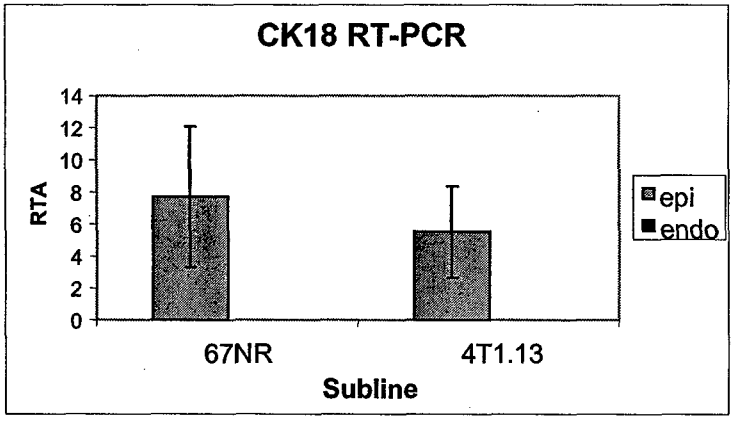
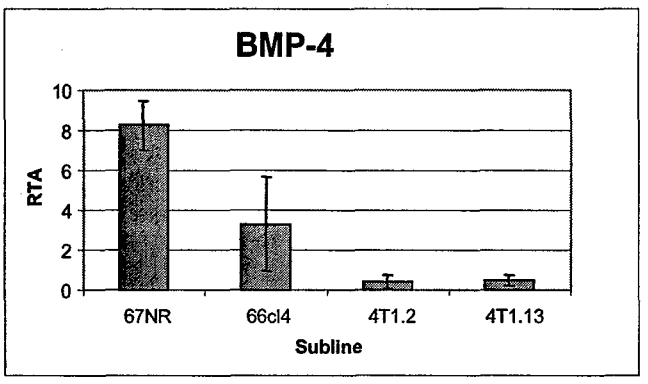
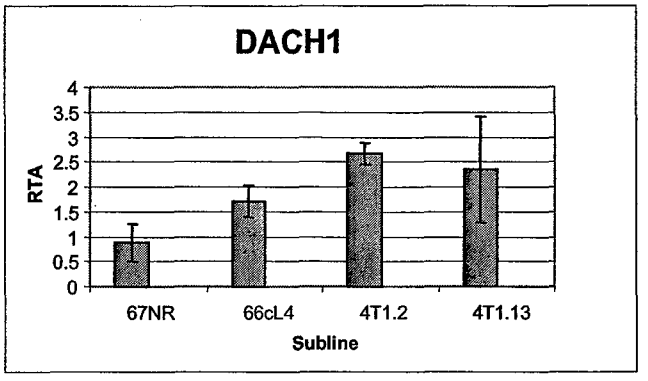


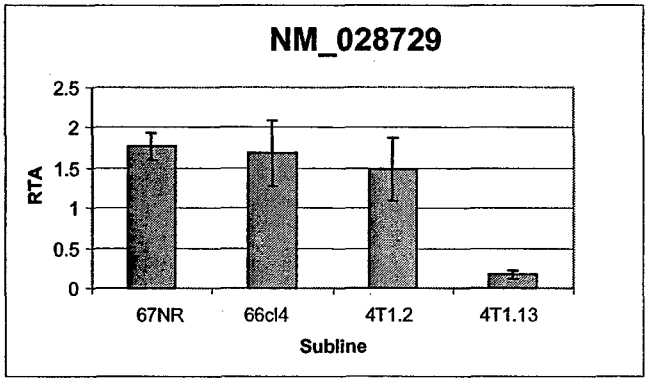

*Figure 2 Verification of endothelial cell identity*
To verify the specific binding of P1H12 to vascular endothelium a number of methods were used. These included immunohistochemistry on human foreskin (A) and mouse hind skin (data not shown) and RT-PCR of immunopurified populations of epithelial and endothelial populations with genes specific for endothelium (vWF, P1H12) and epithelium (CK18), shown in panels B and C respectively.



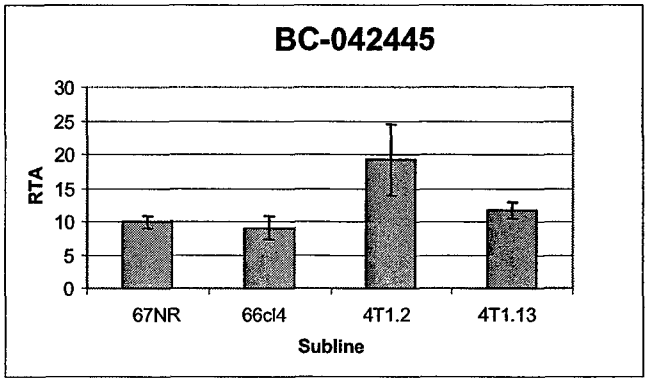

Microarray= Decreased 2.5 fold 4T1.2, 4T1.13 v's 67NR, 66cl4 in epithelium




Microarray= Increased 2.4 fold high met epithelium




Microarray = 2.2-fold decrease epi specific

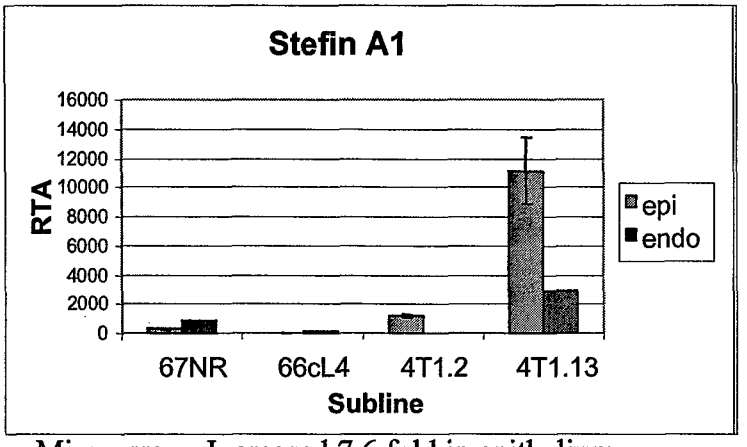



Microarray = 1.9-fold increase epi specific

***Figure 3 Genes with altered expression in highly metastatic primary epithelium***

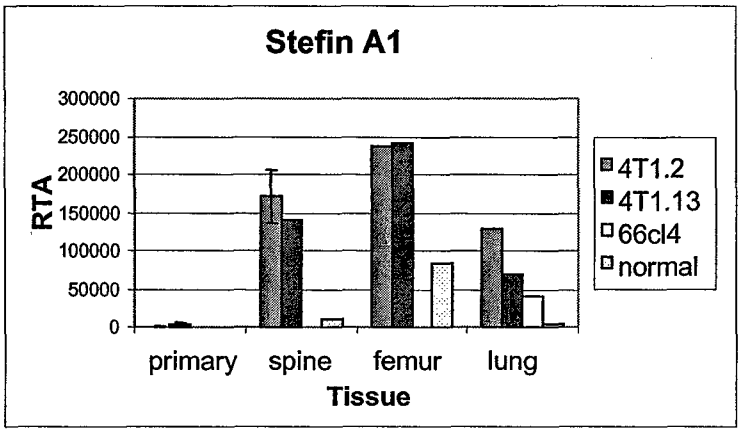
Genes (BMP-4, DACH1) and ESTs (NM_028729, BC-042445) found differentially expressed by microarray in epithelial cells immunopurified from highly metastatic primary tumors were verified by quantitative RT-PCR. RNA samples from 5 duplicate immunopurified samples from primary tumors of each subline were reverse transcribed using Qiagen Sensiscript RTase. Quantitative RT-PCR was performed using SYBR green and gene specific primers, and GAPDH as a control for normalization. RTA represents the relative transcript abundance when CT values for each gene were normalised to GAPDH. Fold-difference between epithelium derived from high metastatic and low metastatic primary tumors as determined by microarray is indicated below each graph.

A




Microarray= Increased 7.6 fold in epithelium

B




Microarray= increased 9-fold in spine epithelium compared
To primary epithelium

***Figure 4 Expression of Stefin A1 (Stfa1) in primary and metastatic tumors.***
Stefin A1 was found over-expressed in highly metastatic primary tumor epithelium and at even higher levels in epithelium isolated from the spine of mice containing bone metastases. This was verified by using quantitative RT-PCR, revealing an increase in transcript level in highly metastatic sublines at the primary site (A), and a further increase in metastatic lesions (B). 4T1.2 spine epithelium was derived from 6 duplicates, as were all primary epithelial samples. Comparisons to microarray results are shown below each graph.

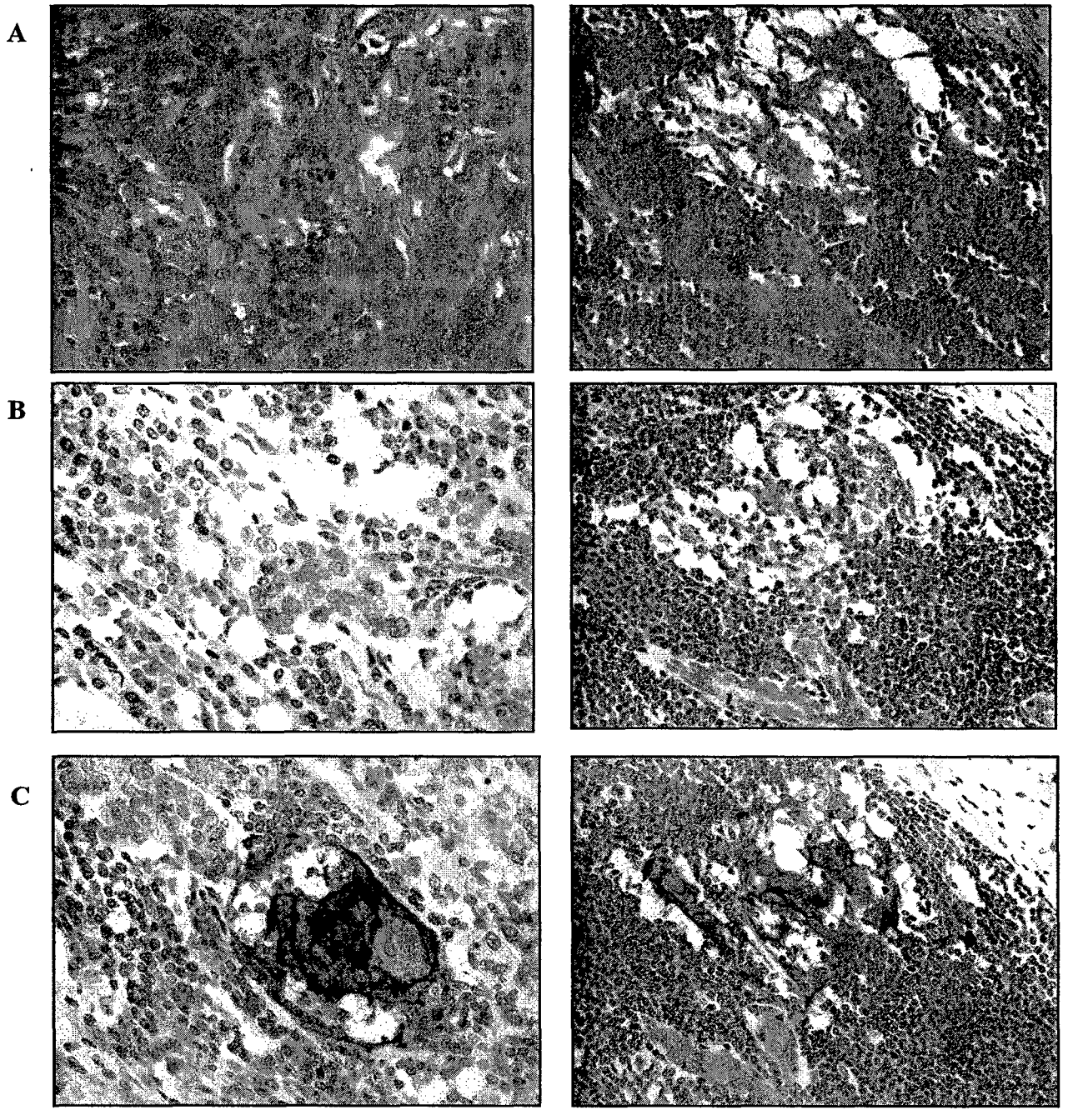



*Figure 5 Immunohistochemistry of Stefin A in human breast cancer*
2 regions of a primary breast tumor and shown stained with hemotoxylin and eosin as a reference (A), IgG negative control (B) and the anti-human stefin A antibody.

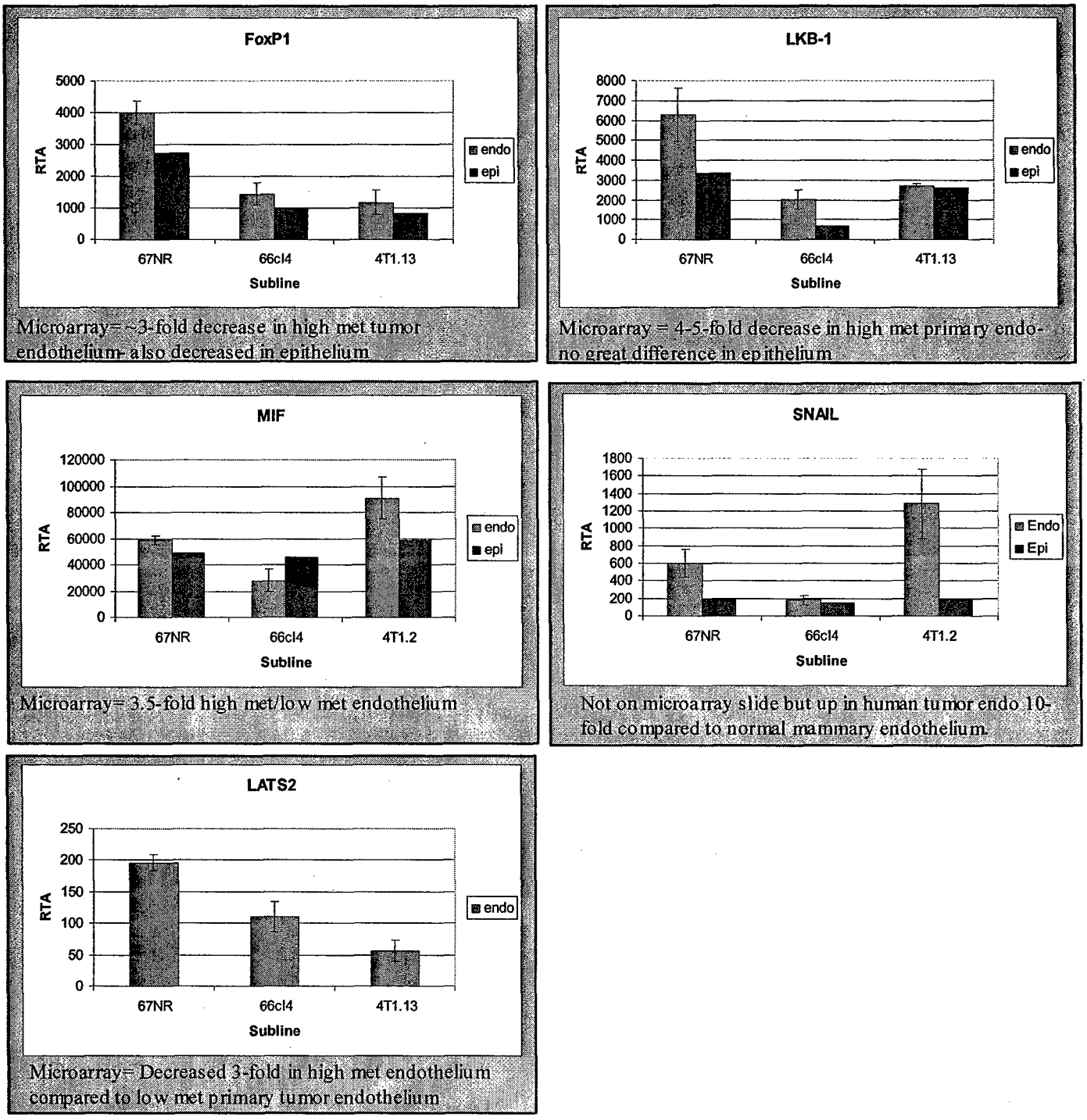



***Figure 6. Genes with altered expression in highly metastatic primary endothelium***
FoxP1, LKB1, MIF, LATS2 and SNAIL found to be differentially expressed by microarray in endothelial cells immunopurified from highly metastatic primary tumors, were verified by quantitative RT-PCR. RNA samples from 5 duplicate immunopurified samples from primary tumors of each subline were reverse transcribed using Qiagen Sensiscript RTase. Fold-difference between endothelium derived from high metastatic and low metastatic primary tumors as determined by microarray is indicated below each graph.

| Acession ID | Description | Common | P-value | High met/ normal | High met/ low met | normal/ high met | Low met/ high met | Function |
|---|---|---|---|---|---|---|---|---|
| BG077636 | endothelial-derived gene | Eg1 | 2.60E-04 | 3.01 | 1.83 | | | angiogenesis |
| BG075854 | AXL receptor tyrosine kinase | Axl | 0.008018 | 9.01 | 2.16 | | | angiogenesis, cell adhesion and proliferation |
| BG087383 | cathepsin D | Ctsd | 0.014202 | 7.08 | 1.98 | | | angiogenesis, ECM degradation |
| BG077749 | macrophage migration inhibitory factor | Mif | 5.49E-04 | 4.64 | 3.49 | | | angiogenesis, endo growth and migration |
| BG069647 | ornithine decarboxylase, structural | odc | 0.014948 | 3.12 | 1.96 | | | tumor invasion/angiogenesis/endo proliferation (supression of endostatin) |
| BG074926 | transducer of ERBB2, 2 | Tob2 | 0.005109 | | | 5.49 | 3.29 | antiproliferative/inhibits cell cycle progression |
| BG072463 | H1 histone family, member 0 | H1f0 | 0.006185 | 3.06 | 2.5 | | | DNA replication/progression through cell cycle |
| C87546 | serine/threonine kinase 11 | Stk11 | 0.004594 | | | 4.39 | 5.2 | tumor suppressor/cell cycle blockage/P53 apoptotic regulation |
| BG069799 | a disintegrin and metalloprotease domain 8 | ADAM8 | 9.82E-05 | 53.47 | 1.92 | | | cell adhesion/growth/osteoclast maturation |
| BG066967 | RAB20, member RAS oncogene family | Rab20 | 0.002162 | 8.33 | 0.85 | | | cell adhesion/migration |
| AW544628 | integrin beta 1 (fibronectin receptor beta) | Itgb1 | 0.001559 | 2.92 | 2.09 | | | cell adhesion/spreading |
| BG088280 | cell adhesion molecule-related/down-regulated by oncogenes | Cdon | 0.00251 | 3.36 | 1.66 | | | cell adhesion |
| BG082041 | RAP2B, member of RAS oncogene family | Rap2b | 0.004098 | 4.94 | 1.34 | | | chemotaxis/cell adhesion, morphology, motility/migration |
| AW542365 | villin 2 | Vil2 | 0.010279 | | | 3.14 | 2.47 | cytokeleton architecture |
| BG077548 | LIM and SH3 protein 1 | Lasp1 | 1.54E-04 | 3.56 | 1.8 | | | cytoskeleton architecture/cell motility/downstream target of hedgehog |
| BG072801 | S100 calcium binding protein A9 (calgranulin B) | S100a9 | 0.004227 | 14.78 | 1.43 | | | transendothelial migration/adhesion to ECM |
| BG071424 | integral membrane protein 2C | Itm2c | 0.003416 | 3.91 | 1.3 | | | integral transmembrane protein/adhesion???? |
| BG083236 | phosphatase and tensin homolog | PTEN | 0.013317 | | | 3.984 | 2.68 | tumor suppressor/proliferation/migration |
| BG076288 | forkhead box P1 | Foxp1 | 0.004227 | | | 3.69 | 2.98 | growth suppression/transcriptional repressor |
| BG076991 | suppressor of cytokine signaling 3 | Socs3 | 0.007995 | 4.17 | 1.47 | | | inhibition of antiproliferative proteins/inhib T cell recognition |
| BG085276 | peroxiredoxin 5 | prdx5 | 1.62E-04 | 6.77 | 2.86 | | | antioxidant/ protective and proliferative role |
| BG088394 | selenoprotein P, plasma, 1 | Sepp1 | 8.17E-05 | 9.67 | 1.3 | | | oxidative stress response |
| AW553304 | BTB and CNC homology 2 | Bach2 | 0.017182 | | | 11.63 | 2.17 | oxidative stress/reduced proliferation/spontaneous cell death |
| BG077758 | histidine triad nucleotide binding protein | Hint | 0.011782 | 2.88 | 1.45 | | | protection against oxidative stress/cell growth |
| BG085960 | apolipoprotein C-II | Apoc2 | 1.62E-04 | 4.72 | 3.16 | | | protection of cells from damage by LDL |
| BG069532 | Niemann Pick type C2 | Npc2 | 8.55E-06 | 6.25 | 1.47 | | | cholesterol homeostasis (like apoC2) |
| BG064823 | phosphoglycerate mutase 1 | pgam1 | 0.002216 | 4 | 1.93 | | | glycolytic enzyme/induced by hypoxia |
| BG084568 | tubulin cofactor a | tbc a | 0.005996 | 2.44 | 1.18 | | | tubulin production/microtubule maintenance |
| BG073029 | CREBBP/EP300 inhibitory protein 1 | Cri1 | 5.51E-04 | 2.7 | 1.21 | | | acetylase/transcriptional activator |
| C87464 | tumor protein D52 | tpd52 | 4.34E-06 | 3.84 | 0.96 | | | signalling intermediate |
| BG068028 | SWI/SNF related, matrix associated, actin dependent regulato | Smarca5 | 0.005919 | | | 3.73 | 1.99 | decreased expression = cell specific differentiation |
| | | | | | | | | |
| BG078674 | cathepsin B | Ctsb | 0.001984 | 6.34 | 0.69 | | | invasion- but not met specific- up in all primary tumor endothelium |

***Table 1 Genes differentially expressed in endothelium of highly metastatic primary tumors compared to normal mammary gland endothelium or endothelium from tumors with low metastatic capacity***

| Accession ID | Description | P-value | High met/ normal | High met/ Low met | Low met/ high met | normal/ high met | Human homolog |
|---|---|---|---|---|---|---|---|
| AW544006 | Mus musculus cDNA clone C0176G03 3', mRNA sequence | 0.0065 | 5.56 | 3.92 | | | Homo sapiens BAC clone RP11-372K14 |
| BG065803 | RIKEN cDNA E230006M18 gene | 0.004469 | 2.93 | 2.17 | | | Homo sapiens 3 BAC RP11-630D12* |
| BG066117 | hypothetical protein 5230400M03 | 1.90E-04 | 8.23 | 1.85 | | | Human DNA sequence from clone RP11-14E8* |
| BG071783 | DNA segment, Chr 9, Wayne State University 18, expressed | 0.006698 | 2.53 | 1.44 | | | Homo sapiens acid cluster protein 33, mRNA |
| BG088499 | hypothetical protein 4732466J24 | 4.14E-04 | 3.1 | 1.15 | | | Homo sapiens endonuclease G-like 1 (ENDOGL1), mRNA |
| BG072866 | Mus musculus, clone MGC:28609 IMAGE:4218551, mRNA, complete cds | 1.19E-05 | 7.15 | 0.8 | | | Homo sapiens 3q BAC RP11-396A13* |
| BG072106 | RIKEN cDNA 9530027K23 gene | 1.60E-05 | | | 1.42 | 3.14 | Homo sapiens hypothetical protein FLJ21918, mRNA |
| BG086714 | RIKEN cDNA 1200013B22 gene | 0.005894 | | | 1.44 | 2.99 | Homo sapiens likely ortholog of rat SNF1/AMP-activated protein kinase (SNARK) |
| BG071241 | RIKEN cDNA 9430029L20 gene | 0.013949 | | | 1.55 | 3.03 | Homo sapiens Kruppel-like factor 13 (KLF13), mRNA |
| BG082241 | DNA segment, Chr 8, ERATO Doi 812, expressed | 0.013024 | | | 1.68 | 3.08 | Homo sapiens hypothetical protein FLJ20244, mRNA |
| AU022584 | RIKEN cDNA 2810489L22 gene | 0.00104 | | | 2.23 | 12.01 | Homo sapiens structural maintenance of chromosomes 6-like 1 (SMC6L1), mRNA |
| BG082333 | RIKEN cDNA 2810489L22 gene | 0.007995 | | | 2.37 | 3.77 | Homo sapiens structural maintenance of chromosomes 6-like 1 (SMC6L1), mRNA |
| AU022611 | RIKEN cDNA 2810489L22 gene | 0.010697 | | | 2.48 | 15.32 | Homo sapiens structural maintenance of chromosomes 6-like 1 (SMC6L1), mRNA |
| BG068755 | ESTs, Moderately similar to T14792 hypothetical protein DKFZp586G0322.1 [H.sapiens] | 0.0028 | | | 2.51 | 3.97 | Homo sapiens protein associated with Myc (PAM), mRNA |
| BG075419 | hypothetical protein LOC219024 | 0.016369 | | | 2.8 | 3.02 | Homo sapiens APEX nuclease (multifunctional DNA repair enzyme) (APEX) gene |
| BG072604 | Mus musculus mRNA, up-regulated by FUS-ERG, 3' region, cDNA fragment: C14G220. * | 0.005797 | | | 3.11 | 4.15 | Homo sapiens large tumor suppressor, homolog 2 (Drosophila) (LATS2), mRNA |
| BG084366 | RIKEN cDNA 2810452K22 gene | 0.002249 | | | 4.62 | 5.59 | Homo sapiens cyclin-dependent kinase 2-interacting protein, mRNA |
| BG078034 | expressed sequence AI120141 | 0.002216 | | | 4.68 | 14.06 | Homo sapiens serine/threonine kinase 17b (apoptosis-inducing) (STK17B), mRNA |
| | | | | | | | *short homologous region <50 bp |

***Table 2 Analysis of genes differentially expressed in endothelium of highly metastatic primary tumors compared to normal mammary gland endothelium or endothelium from tumors with low metastatic capacity.*** The corresponding human homologs of these ESTs are shown.

| Acession ID | Description | Common | P value | high met/ low met | low met/ high met | Function |
|---|---|---|---|---|---|---|
| BG074171 | stefin A1 | stfa1 | 0.007 | 7.6 | | cystatin, inhibitor of Cathepsin S, decrease antigen processing and presentation |
| BG085518 | ets variant gene 6 (TEL oncogene) | ETV6 | 0.004 | 7.2 | | cellular aggregation, transformation |
| BG072171 | histidine decarboxylase | Hdc | 0.003 | 5.8 | | synthesis of histamine, tumor cell proliferation |
| BG064722 | SMT3 (supressor of mif two, 3) homolog 1 (S. cerevisiae) | Smt3h1 | 0.001 | 3.9 | | protection against TNF etc cell death, cell cycle progression |
| BG064723 | SMT3 (supressor of mif two, 3) homolog 1 (S. cerevisiae) | Smt3h1 | 3.00E-04 | 3.9 | | protection against TNF etc cell death, cell cycle progression |
| BG087429 | complement component 3 | C3 | 0.004 | 3.8 | | up in cancer patients, including highly metastatic human melanoma cell line |
| BG072394 | histidine decarboxylase | Hdc | 0.003 | 3.5 | | synthesis of histamine, tumor cell proliferation |
| BG071895 | developmental pluripotency associated 5 | dppa5 | 7.00E-04 | 3 | | development |
| BG082584 | envoplakin | Evpl | 0.008 | 2.6 | | cytolinker protein |
| BG079103 | histone cell cycle regulation defective homolog A (S. cerevisiae) | Hira | 0.007 | 2.5 | | Histone chaperone, nucleosome assembly, cell cycle control |
| BG075820 | dachshund 1 (Drosophila) | DACH1 | 0.004 | 2.4 | | cell proliferation, inhibits apoptosis (TGFB), developmental gene |
| BG077636 | endothelial-derived gene | Eg1 | 0.006 | 2.4 | | angiogenesis (originally identified in tumor endothelium-also found in tumor epithelium) |
| BG066360 | Rho GTPase activating protein 8 | Arhgap8 | 0.004 | 2.3 | | cytoskeletal organization, cell cycle regulation, ras-mediated oncogenic transformation |
| BG071729 | maternal effect gene | Mater | 0.004 | 2.2 | | early development |
| BG065738 | Rho guanine nucleotide exchange factor (GEF) 3 | Arhgef3 | 0.008 | 1.8 | | cytoskeletal rearrangement |
| BG065879 | eomesodermin homolog (Xenopus laevis) | Eomes | 0.006 | 1.8 | | early developmental gene |
| BG071892 | RAD21 homolog (S. pombe) | Rad21 | 0.008 | 1.8 | | up in prostate cancer, chromatin cohesion protein |
| BG088006 | signal transducer and activator of transcription interacting protein 1 | statip1 | 0.01 | 1.8 | | cytokine signalling, stat3 interacting protein, stat 3 has role in oncogenesis |
| BG081111 | topoisomerase (DNA) I | Top1 | 0.001 | | 2.1 | realxation of supercolied DNA through breakage, cell cycle checkpoint |
| BG077363 | breast cancer metastasis-suppressor 1 | Brms1 | 0.002 | | 2.2 | Supression of metastasis, function not clear, may be involved in transcrip.regulation |
| BG064270 | mitogen activated protein kinase 1 | MAPK1 | 0.005 | | 2.4 | activation of ERK1/ERK2, roles in differentiation |
| BG064129 | golgi SNAP receptor complex member 1 | Gosr1 | 0.003 | | 2.5 | trafficking membrane protein b/n ER and golgi |
| BG078804 | bone morphogenetic protein 4 | BMP4 | 6.00E-05 | | 2.5 | member of TGF-beta superfamily, induce cell senescence |
| BG064070 | ARP3 actin-related protein 3 homolog (yeast) | ARP3 | 0.006 | | 2.6 | actin nucleation, organization of the cytoskeleton, controls polarised cell growth |
| BG085457 | MAP kinase-interacting serine/threonine kinase 2 | Mknk2 | 0.003 | | 2.6 | limit/inhibit translation |
| BG077621 | lamin A | Lmna | 0.006 | | 2.7 | exp. correlated with non-prolif. cells and tissues, nuclear intermediate filament protein |
| BG087426 | heat shock protein 8 | HSP8/73 | 0.005 | | 2.8 | chaperone |
| BG063869 | autophagy 7-like (S. cerevisiae) | Apg7l | 0.002 | | 3 | enzyme essential for autophagy |
| BG082615 | DnaJ (Hsp40) homolog, subfamily B, member 9 | Dnajb9 | 0.003 | | 3 | stress induced, induced upon anti-metastatic activity (in lung adenocarcinoma cells) |
| BG077186 | heat shock protein 8 | HSP8/73 | 0.01 | | 3 | chaperone |
| BG064223 | DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 21 (RNA helicase | Ddx21 | 4.00E-04 | | 3.1 | rRNA biogenesis |
| BG065503 | integrin beta 5 | Itgb5 | 0.008 | | 3.1 | cell-ECM interaction |
| BG088147 | zinc finger protein X-linked | Zfx | 0.004 | | 3.1 | inhibits angiogenesis, involved in embryonic growth (sex differentiation) |
| BG081794 | similar to Mid-1-related chloride channel 1 | Mclc | 0.007 | | 3.2 | roles in apoptosis and cell growth |
| BG068990 | suppressor of Ty 6 homolog (S. cerevisiae) | supt6h | 2.00E-04 | | 3.7 | regulate transcription through maintenance of chromatin structure |
| BG080311 | ATP-binding cassette, sub-family B (MDR/TAP), member 1B | Abcb1b | 0.006 | | 4 | ABC protein, membrane transporter |
| BG085841 | nuclear factor, erythroid derived 2, like 2 | Nfe2l2 | 0.002 | | 4.6 | transcription factor, differentiation |

*Table 3 Genes differentially expressed in highly metastatic primary tumor epithelium compared to tumors with low metastatic capacity*

| Gene | Up/down | Human chrom. location | LOH/gene amplification | Function/Cancer association |
|---|---|---|---|---|
| Stefin A1 | Up | 3q21 | LOH | -Cystatin, inhibitor of Cathepsin S (antigen presentation)<br>-Marker of malignancy in some experimental tumors |
| Smt3h1/ SUMO | Up | 21q22.3 | gene amp | -protection against TNF cell death<br>- interacts with MDM2<br>-cell cycle progression |
| Dach 1 | Up | 13q22 | LOH & gene amp | -Cell proliferation, inhibits TGFβ-induced apoptosis<br>-Developmental gene<br>-Role in skeletal development (stimulated by FGF and inhibited by **BMP4**) |
| EST (BC-042445) | Up | 15q21.3 | Allelic imbalance | -no known function<br>- homology to human KIAA1584 protein (AB046804) |
| Lamin A | Down | 1q21.2-21.3 | LOH | -nuclear intermediate filament protein<br>-expression correlated with non-prolif. cells and tissues<br>- apoptotic protein |
| BMP4 | Down | 14q22-23 | LOH | -member of TGF-beta superfamily<br>- developmental role, induces cell senescence<br>- may have a role in hedgehog signalling |
| EST (NM_028729) | Down | 11q13.5 | LOH & gene amp | - no known fucntion<br>- homology to human hypothetical protein FLJ25416 (BC039268) |

***Table 4: List of candidate genes aberrantly expressed in highly metastatic primary tumor epithelium.*** Genes that are increased (up) or decreased (down) in the highly metastatic tumors (4T1.2, 4T1.13) compared to those that do not metastasize to bone are indicated, along with the human chromosomal localization and known gene functions and previous documentation in cancer. Also included is past evidence of LOH or gene amplification in that chromosome region in breast cancer.

## KEY RESEARCH ACCOMPLISHMENTS

- Purification of epithelial, endothelial and "stromal" cells from primary tumors derived from an *in vivo* model of breast cancer metastasis
- Confirmation of endothelial cell purity using the immunopurification method
- Microarray gene expression profiling of cell specific alterations with increasing metastatic propensity of primary tumors
- Generation of tables of genes statistically different in endothelial and epithelial cells from highly metastatic primary tumors
- Quantitative RT-PCR verification of genes altered in the endothelium (FoxP1, LKB-1, MIF, SNAIL, LATS2) and epithelium (Stefin A1, BMP-4, DACH1, and 2 ESTs)
- Verification of high Stefin A1 expression in epithelial cells purified from bone metastases (higher than that in matched primary tumors)
- Detection of Stefin A expression also in human breast cancer by immunohistochemistry

## REPORTABLE OUTCOMES

Awards

2003 AACR Special Conference Scholar-in-Training Award (provided by the Avon Foundation).

Publications relating to project

Parker, B.S., Eckhardt, B.L. and Anderson, R.L. (2004). Models of breast cancer metastasis to bone: characterization of a clinically relevant model. In Bone Metastasis, Eds. G. Singh and F.W. Orr, Kluwer Press, The Netherlands.

Parker, B.S., Argani, P, Cook, B.P., Liang Fen, H., Chartrand, S.D., Zhang, M., Saha, S., Bardelli, A., Yiang, Y., St. Martin, T.B., Nacht, M., Teicher, B.A., Klinger, K.W., Sukumar, S. and Madden, S.L. (2004). Alterations in vascular gene expression in invasive breast cancer. *Cancer Research*, Accepted.

Conference presentations

Cell specific gene expression profiling in a murine model of breast cancer metastasis. Parker, B.S. and Anderson, R.L. Metastasis Research Society, 10th Int'l Congress, Genoa Italy September 17-20, 2004

Aberrant gene expression in breast cancer endothelium. Parker, B.S., Madden, S.L., Sukumar, S.S. and Anderson, R.L. Advances in Breast Cancer Research: Genetics, Biology, and Clinical Implications (AACR Special Conference), 2003. Huntington Beach, CA, USA.

Aberrant gene expression in breast cancer endothelium . Parker, B.S., Madden, S.L., Sukumar, S.S. and Anderson, R.L. 5th Peter MacCallum Cancer Centre Symposium, November, 2003. Melbourne, Australia.

## CONCLUSIONS

This study has investigated cell specific gene expression alterations during metastatic progression of breast cancer in a clinically relevant *in vivo* model. Endothelial and epithelial cells have been successfully purified from primary breast tumors and (subsequent to RNA amplification and labelling) have been expression profiled using microarray.

A number of candidates have been identified over-expressed or suppressed in tumor endothelium and in the tumor cells themselves during metastatic progression. These candidates have been verified and will be analysed further for their functional role in metastasis, and for their role in human breast cancer.

One such gene, stefin A1, has an enhanced gene expression in tumor cells with greater metastatic propensity and this expression is elevated in matched spine and femur metastases. Importantly, this gene also has relevance in human breast cancer, with detection of protein expression in a subset of primary tumors. This work is going to be continued to determine the prognostic significance of stefin A expression in human breast cancer.

This work has many implications to breast cancer research. The use of a clinically relevant model of breast cancer metastasis was not only useful for finding gene candidates but is also of enormous importance in determining the functional role of such genes in the metastasis process. This has not been available in other studies, and may be responsible for the lack of molecular markers as prognostic indicators and targets for treatment. Additionally, many genes that have previously been associated with human cancer progression have been identified in this study and that fact that we have already verified expression of one of our candidates of interest in human breast cancer reveals the clinical relevance of this model in researching breast cancer metastasis.

## REFERENCES


1) Rubens, R.D., *Bone metastases - the clinical problem.* European Journal of Cancer, 1998. *34*: p. 210-213.
2) Bissell, M.J. and D. Radisky, *Putting tumors in context.* Nature Reviews (Cancer), 2001. *1*: p. 1-19.
3) Cunha, G. R. and Matrisian, L. M. It's not my fault, blame it on my microenvironment. *Differentiation, 70:* 469-472, 2002.
4) Olumi, A. F., Grossfeld, G. D., Hayward, S. W., Carroll, P. R., Tlsty, T. D., and Cunha, G. R. Carcinoma-associated fibroblasts direct tumor progression of initiated human prostatic epithelium. *Cancer Res, 59:* 5002-5011, 1999.
5) Allinen, M., Beroukhim, R., Cai, L., Brennan, C., Lahti-Domenici, J., Huang, H., Porter, D., Hu, M., Chin, L., Richardson, A., Schnitt, S., Sellers, W.R., and Polyak, K. *Cancer Cell, 6:* 17-32, 2004.
6) Lelekakis, M., Moseley, J. M., Martin, T. J., Hards, D., Williams, E., Ho, P., Lowen, D., Javni, J., Miller, F. R., Slavin, J., and Anderson, R. L. A novel orthotopic model of breast cancer metastasis to bone. *Clinical and Experimental Metastasis, 17:* 163-170, 1999.
7) St Croix, B., Rago, C., Velculescu, V., Traverso, G., Romans, K. E., Montgomery, E., Lal, A., Riggins, G. J., Lengauer, C., Vogelstein, B., and Kinzler, K. W. Genes expressed in human tumor endothelium. *Science, 289:* 1197-1202, 2000.
8) Parker, B.S., Argani, P, Cook, B.P., Liang Fen, H., Chartrand, S.D., Zhang, M., Saha, S., Bardelli, A., Yiang, Y., St. Martin, T.B., Nacht, M., Teicher, B.A., Klinger, K.W., Sukumar, S. and Madden, S.L. (2004). Alterations in vascular gene expression in invasive breast cancer. *Cancer Research*, Accepted.

APPENDIX

# CURRICULUM VITAE

PII Redacted


| | |
|---|---|
| Name: | **Belinda Sheree Parker** |
| [redacted] | [redacted] |
| Work Address: | Peter MacCallum Cancer Centre<br>Department of Research<br>St Andrews Place, East Melbourne, 3002 |
| [redacted] | [redacted] |
| Telephone: | (BH) 03 96561306 (AH) 0402 849305 |
| Fax: | 03 96561411 |
| Email: | belinda.parker@petermac.org |
| [redacted] | [redacted] |


## TERTIARY QUALIFICATIONS

1998-2001 **PhD** (submitted 23/08/001), Dept. of Biochemistry, La Trobe University
Supervisors : Dr D.R.Phillips and Dr S. M. Cutts

1997 **Honours year** Biochemistry, La Trobe University -Result H1

1994-1996 **Bachelor of Biological Sciences** La Trobe University
Majors: Biochemistry and Human Genetics

## POSTDOCTORAL EXPERIENCE

March 2003-present Postdoctoral Fellow, Department of Research, Peter MacCallum Cancer Centre.
Field of Study- Breast Cancer
PI: Dr. Robin Anderson

Nov 2001-March 2003 Postdoctoral Fellow,Department of oncology, Johns Hopkins University, MD, USA.
Field of study- Breast Cancer
PI: Professor Saraswati Sukumar

## AWARDS AND FELLOWSHIPS

2003 AACR Special Conference Scholar-in-Training Award (provided by the Avon Foundation).

2003 Awarded- Department of Defence Breast Cancer Research Program (BCRP) Postdoctoral Fellowship.
(July 2003-July 2006)
- Susan Komen Postdocoral Fellowship (declined)
- Peter Doherty Postdoctoral Fellowship (March 2003-July 2003 then declined)

2000 Australian Society of Biochemistry and Molecular Biology (ASBMB) travel Fellowship.

1998 LaTrobe University Postgraduate scholarship

## SCIENTIFIC PUBLICATIONS

Parker, B.S., Eckhardt, B.L. and Anderson, R.L. (2004). Models of breast cancer metastasis to bone: characterization of a clinically relevant model. In Bone Metastasis, Eds. G. Singh and F.W. Orr, Kluwer Press, The Netherlands.

Parker, B.S., Rephaeli, A., Nudelman, A., Phillips, D.R. and Cutts, S.M. (2004). Formation of mitoxantrone adducts in human tumor cells: Potentiation by AN-9 and DNA methylation. *Oncology Research.* In press.

Parker, B.S., Buley, T., Evison, B.J., Cutts, S.M., Neumann, G.M., Iskander, M.N. and Phillips, D.R. (2004). A molecular understanding of mitoxantrone-DNA adduct formation: Effect of cytosine methylation and flanking sequences. *J. Biol. Chem.* In Press (Epub).

Parker, B.S., Argani, P, Cook, B.P., Saha, S., Fen, H.L., Bardelli, A., Madden, S.L, Sukumar, S. (2004). Aberrant expression of genes in human breast tumor vascular endothelium: Genetic profiling and validation. Submitted.

Waly, M., Olteanu, H., Banerjee, R., Choi, S.-W., Mason, J.B., Parker, B.S., Sukumar, S., Shim, S., Sharma, A., Benzecry, J.M., Power-Charnitsky, V.-A. and Deth, R.C. (2004). Activation of methionine synthase by insulin-like growth factor-1 and dopamine: A target for neurodevelopmental toxins and thimerosal. *Molecular Psychiatry.* In Press (Epub).

Parker, B.S., Cutts, S.M., Nudelman, A., Rephaeli, A., Phillips, D.R. and Sukumar, S. (2003). Mitoxantrone mediates Demethylation and re-expression of cyclin D2, estrogen receptor and 14-3-3σ in breast cancer cells. *Cancer Biol. & Ther.* 2(3), 259-263.

Parker, B.S. and Sukumar, S. (2003). Distant metastasis in breast cancer: Molecular mechanisms and a search for therapeutic targets. *Cancer Biol. & Ther.* 2(1), 14-21.

Parker, B.S., Cutts, S.M. and Phillips, D.R. (2001). Cytosine methylation enhances mitoxantrone-DNA adduct formation at CpG dinucleotides. *J. Biol. Chem.* 276(19), 15953-15960.

Cutts, S.M., Parker, B. S., Swift, L. P., Kimura, K. and Phillips, D.R. (2000). Structural requirements for the formation of anthracycline-DNA adducts. *Anticancer Drug Design.* 15(5), 373-386.

Parker, B.S., Cutts, S.M., Cullinane, C. and Phillips, D.R. (2000). Formaldehyde activation of mitoxantrone yields CpG and CpG specific DNA adducts. *Nucleic Acids Research* 28, 982-990.

Parker, B.S., Cullinane, C. and Phillips, D.R. (1999). Formation of DNA adducts by formaldehyde-activated mitoxantrone. *Nucleic Acids Research* 27, 2918-2923.

## ONGOING RESEARCH SUPPORT

DOD/BCRP - DAMD17-03-1-0473
Stromal gene expression in primary breast tumors that metastasize to bone. July 2003-July 2006
Role: PI

## CONFERENCE PRESENTATIONS

Aberrant gene expression in breast cancer endothelium . Parker, B.S., Madden, S.L., Sukumar, S.S. and Anderson, R.L. 5th Peter MacCallum Cancer Centre Symposium, November, 2003. Melbourne, Australia.

Aberrant gene expression in breast cancer endothelium. Parker, B.S., Madden, S.L., Sukumar, S.S. and Anderson, R.L. Advances in Breast Cancer Research: Genetics, Biology, and Clinical Implications (AACR Special Conference), 2003. Huntington Beach, CA, USA.

Enhancement of drug-DNA binding at methylated cytosine residues by the CpG specific anticancer drug mitoxantrone. Parker, B. S., Cutts, S. M. and Phillips, D.R. Annual Conference of the American Association of Cancer Research, March 2001. New Orleans, LS, USA.

The role of CpG methylation on mitoxantrone-DNA adduct formation. Parker, B.S., Swift, L. P., Cutts, S. M. and Phillips, D.R. Australian Society of Biochemistry and Molecular Biology Annual Conference, December 2000. Wellington, New Zealand.

Formaldehyde mediated DNA alkylation by mitoxantrone. Parker, B.S., Cullinane, C. and Phillips, D.R. Molecular Determinants of Sensitivity to Antitumour Agents, American Association of Cancer Research, March, 1999. Whistler, Canada

Molecular and cellular studies of the activation of mitoxantrone by formaldehyde. Parker, B.S., Cullinane, C. and Phillips, D.R. 11th Lorne Cancer Conference, Feb, 1999. Lorne, Australia

## RELEVANT EXPERIENCE

| | |
|---|---|
| Aug 2001-Nov 2001 | Casual senior research assistant, Biochemistry Department, La Trobe University |
| 1998-2001 | Demonstrating 2nd and 3rd year biochemistry practical classes |

## REFEREES

**Dr. Robin Anderson**
Head, Cancer Biology
Department of Research
Peter MacCallum Cancer Center
St Andrews Place
East Melbourne, 3002
Phone: 61 (0)3 96561286
Email: robin.anderson@petermac.org

**Professor Don R. Phillips**
Reader in Biochemistry
La Trobe University
Bundoora, 3086
Australia
Phone: 61-3-9479 2182
Email: D.Phillips@latrobe.edu.au

**Prof. Sara Sukumar**
Professor of Oncology
Sidney Kimmel Comprehensive
Cancer Center at Johns Hopkins
1650 Orleans St
Baltimore, MD, 21231, USA
Phone: 11 410 6142479
Email: sukumsa@jhmi.edu

**Dr. Suzanne M. Cutts**
Research Associate
School of Biochemistry
La Trobe University
Bundoora, 3086
Australia
Phone: 61-3-9479 1182
Email:

scutts@bioserve.latrobe.edu.au